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It has been postulated that the heterogeneous, metastatic breast cancer cells are originated from a single or a small number of distinct tumor stem cells, and that only the cancer stem cells have the unlimited ability to proliferate and metastasize to different tissues and organs. A recent study found that the CD44+/CD24- breast tumor cells may be breast tumor stem cells. CD44 is a receptor that binds to hyaluronan (HA), a carbohydrate consisting of ß1,3 *N*-acetyl glucosaminyl-ß1,4 glucuronide. We have proposed to generate CD44-targeted liposome to selectively destroy the CD44+ breast tumor cells. Toward the goal, we have prepared HA molecules from human umbilical cord hyaluronic acid by hydrolysed by Bee venom. However, we have encountered the technical difficulty to produce CD44-targeted liposomes that are incorporated with HA molecules. Due to the technical problems, this proposed study has been extended for additional one year.

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USAMRMC FY02 BREAST CANCER RESEARCH PROGRAM

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It has been postulated that the heterogeneous, metastatic breast cancer cells are originated from a single or a small number of distinct tumor stem cells, and that only the cancer stem cells have the unlimited ability to proliferate and metastasize to different tissues and organs. Such a breast tumor stem cell population was recently identified (1). It was found that only one cell type with high levels of CD44 and low levels of CD24, about 1% to 15%, of breast cancer cells removed from patients were capable of forming new malignant tumors in the nude mice (mammary glands). As few as 100 to 200 of these CD44+/CD24- breast tumor cells readily formed tumors in mice, while tens of thousands of other tumor cells from the same tumor failed to grow, suggesting that the CD44+/CD24- breast tumor cells may be breast tumor stem cells (1). CD44 is a receptor that binds to hyaluronan (HA), a carbohydrate consisting of ß1,3 N-acetyl glucosaminyl-ß1,4 glucuronide (2). CD44 is found at low levels on a number of cell types in normal tissues, including epithelial, hemopoietic, and neuronal cells. However, these normal cells are either not in direct contact with the blood or require activation before they bind to HA (2). Thus, CD44 may be a suitable surface marker for targeted killing of breast tumor stem cells that express a high level of this molecule.

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The goal of this study is to generate CD44-targeted liposome to selectively destroy the CD44+ breast tumor cells.

Task 1. To generate and characterize CD44-targeted liposome that is incorporated with HA. We have prepared HA molecules from human umbilical cord hyaluronic acid by hydrolysed by Bee venom. Hyaluronic acid fragments were separated on 11 x 265 mm column of the formate form of Bio-Rad AG-3 x 4A ion-exchange resin. However, we have encountered the technical difficulty to produce CD44-targeted liposomes that are incorporated with HA molecules. Due to the technical problems, this proposed study has been extended for additional one year.

We are using the method described by Szoka's laboratory (*Cancer Research* 61, 2592-2601, 2001) to prepare HA-incorporated liposome. Briefly, Chol (Sigma Co. (St. Louis)) was recrystallized from methanol. Lipid films were prepared by drying 10 μmol of lipid including POPE-HA or DPPE-HA from solvent (butanol saturated with distilled water or chloroform:methanol 7:3, respectively) under vacuum using rotary evaporator at room temperature. Liposomes (composed of POPC:Chol:HA-POPE 60:40:3) were prepared by rehydrating the lipid film with 1 ml of 10 mM HEPES, 5% glucose (pH 7.4), followed by mixing on a vortex mixer for 1 min, sonication for 15 min in a bath type sonicator (Laboratory Supplies Company Inc., Hicksville, NY).

The problem we encountered is that the sizes of HA-liposomes we generated were mostly larger than 0.5 μm, which was determined by light scattering analyses (N4 particle size analyzer, Coulter). The sizes of HA-liposomes are too big to be biologically active (should be in the range of 0.1-0.2 μm). Currently, we are modifying the protocol by testing different experimental conditions in order to achieve a smaller size of liposomes.

Task 2. To evaluate if HA-targeted liposomes (HALs) can selectively bind to the breast cancer cells expressing high levels of CD44.
To be performed

Task 3. To evaluate if HA-targeted, drug-containing liposomes (HALs) can selectively kill the breast cancer cells expressing high levels of CD44.
To be performed

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Experiments are under way to accomplish the Task 1.

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Experiments are under way to accomplish the Task 1.

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Experiments are under way to accomplish the Task 1.